SpotScout

User Manual

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Dear customer

We are delighted that have chosen ***SpotScout*** or ***SpotScout Pro***, a Scan-Software to control the ***Microarray Scanner MArS*** of ***Ditabis*** and to qualify and evaluate biochip image data.
The Software has been developed with utmost care involving the use of newest tools and high-performance algorithms to allow routine operation as well as individual research tasks.
The intuitive user navigation makes working with the ***SpotScout*** (***Pro***) very simple. The operator's manual includes all that you need for operating the software and for using all implemented functions.

We wish you every success in solving your tasks with ***SpotScout (Pro)***. If you have any questions or if a problem occurs, please do not hesitate to contact us.

Your Ditabis AG Development Team

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75179 Pforzheim
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1. Overview

1.1. What is *SpotScout* and *SpotScout Pro*

SpotScout is the basic module of the scanner software to control the ***MArS*** Microarray Scanner and to display biochip image data for analysis. It is also possible to load and display image data of other devices if they are available as 16 bit grey scale images in TIF format. Besides, it is possible to import all GAL files.

SpotScout Pro additionally includes the complete evaluation module to evaluate image data automatically or pursuant to individual specifications:

- Automatic grid finding
- Quantification of image data
- Reliable and efficient quality control (spot position evaluation, artifact identification, saturation check) with report
- Export of the results in GPR format

With the evaluation module of ***SpotScout Pro*** you can also evaluate TIF image files.

Definition of Terms
To simplify matters, we talk about the software system ***SpotScout*** in this operator's manual if functions are concerned which exist in both systems. ***SpotScout Pro*** is used if functions are concerned existing only in this system.

1.2. System Requirements

Hardware	
Processor	2GHz (multi-core recommended)
Main Memory	at least 2GB (4GB recommended)
	USB 1.0
Software	
Operating System	Windows XP – Win7 (only 32bit!)
	Acrobat 6.0 or higher
	MArS with firmware 1.0.32 or higher

During a scan, no other software besides SpotScout may be running.

1.3. Program Version

Version 1.

1.4. Content and Structure of the Manual

The available manual is the operating manual and software documentation for ***SpotScout*** and ***SpotScout Pro***. Since ***SpotScout*** is a subset of ***SpotScout Pro***, it is indicated which software functions surpass the basis module ***SpotScout***. Basically, it is the menu **Evaluation**.

The manual is structured in such a way that every user can immediately find “his” parts.

Chapter 1 includes an overview over the software, explains the writing rules and provides license information.

Chapter 2 describes the installation of the software and the connection with the Microarray Scanner MArS as well as the activation of the Software with the license key.

Chapter 3 represents the Software documentation. Here, the individual functions and their meaning are described.

Chapter 4 is the appendix with further explanations about the program.

1.5. Writing Rules

In the available manual we use the following writing rules for better orientation:

The names of ***Menus***, ***Options***, ***Register Tabs*** etc. are written in bold letters	**File**, **Viewer**, **Protocol**
The ***Selection of an Option in a Menu*** is written as a command sequence, separated by a vertical line	**File | Options**
Buttons, if not depicted, are written in bold letters and set in angle brackets	**<Save>**, **<Open>**, **<Close>**
Enumerations	•
Action steps	❑

1.6. License Information

ZedGraph is published under LGPL
GNU LESSER GENERAL PUBLIC LICENSE
Version 3, 29 June 2007

Copyright (C) 2007 Free Software Foundation, Inc. http://fsf.org/

Please read the license information completely and observe it. It is included in the appendix in chapter 4.2.

2. Software Installation and Connection with MArS

This chapter describes the installation of ***SpotScout*** or ***SpotScout Pro*** on your PC and the connection of the PC with the ***Microarray Scanner MArS*** and the activation of the Software.
You get the Software either on CD-ROM or by download.
After the **Installation of the Software**, the **Software is activated** by receiving a license key from **Ditabis AG**.

Installation Procedure

- ❑ You get the Setup file on the enclosed CD-ROM or have downloaded it.
- ❑ Start the file **setup.exe**.
- ❑ When asked for the target drive, enter the desired path, e.g. **C:\Programme\Ditabis*SpotScout***.
- ❑ If **.Net Framework 4.0** is not installed on your computer it will be automatically installed during the setup.
- ❑ If you connect the **Microarray Scanner MArS** with the PC for the first time you have to install the corresponding USB driver:
- ❑ For that, switch on **MArS**, wait a few seconds and connect the instrument with your PC with an USB cable.
- ❑ The PC now detects a new USB device and Windows requests you to search a corresponding driver in a directory. Select the setup directory or the directory you have downloaded. After that, the USB driver is installed automatically.
- ❑ As last installation step, click on <**Next**> or <**Continue**>. Finally, Windows indicates that the installation has been successful.
- ❑ The setup routine creates a file in the Start menu and the icon on your desktop.
- ❑ For starting ***SpotScout*** or ***SpotScout Pro*** double-click the program icon on your desktop.

Activation of the Software with a License Key - Procedure

- ❑ Select “GetSpotScoutID” in the start menu. Then, the file “HardwareID.txt” is created on your desktop.
- ❑ Send the file “HardwareID.txt” per email to sales@ditabis.de.
- ❑ After having paid the license fee, we will send you the license file immediately. Otherwise, you get information on payment options.
- ❑ Copy the license file in the ***SpotScout*** program directory and start ***SpotScout***.

3. Software Documentation

3.1. Program Structure and Operation

3.1.1. Structure of the Program Window

After the program start (click on the program icon on the desktop), ***SpotScout*** or ***SpotScout Pro*** is loaded and the program with the start window is displayed. This is the window of the menu **Viewer** with the display fields for the images still being empty.
If ***MArS*** is not connected and switched on, all functions are deactivated.

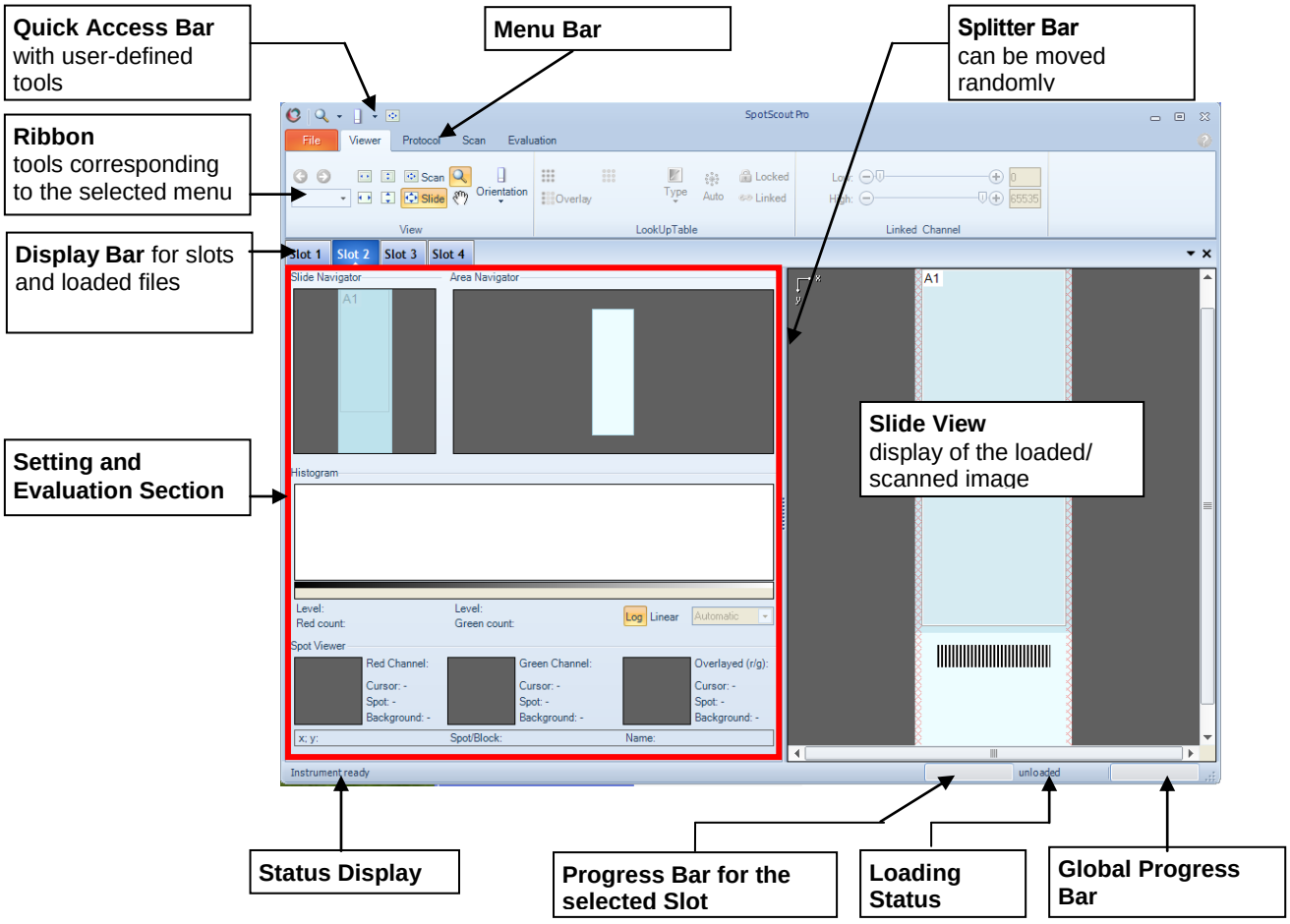


Fig. 1: Structure of the Program Window

In all program parts, the program window is structured similarly. Only in the menu **File** there is no **Slide View**.

3.1.2. Operation

Quick Access

You can copy buttons and tools which you use frequently from the **Ribbon** into the tool bar **Quick Access**. Then, they are additionally available in every menu. The quick access can be configured pursuant to your individual needs. For that, the **Context Menu** of the individual **Buttons** or **Functional Groups** (e.g. **View** or **Lookup Table**) is available. The options of the **Context Menu** are related to the clicked-on object.
The buttons can be removed from the **Quick Access** again by opening the **Context Menu** of the **Button** or the **Functional Group** in **Quick Access** and selecting the relevant option (e.g. **Remove...**).

The options of the context menu

Add to Quick Access Toolbar	Copies the clicked- on function or function group into the Quick Access Bar.
Remove from Quick Access Toolbar	*Reversing:* Right mouse click on the function or the functional group in the Quick Access Bar and select **Remove...**
Customize Quick Access Toolbar…	Via a dialogue window, selected functions of all menus can be selected and copied in **Quick Access** or removed from **Quick Access**.
Place Quick Access Toolbar below/ above the Ribbon	Relocates Quick Access into the bar below or above the **Ribbon**.
Minimize the Ribbon	Fades the ribbon out.
Maximize the Ribbon	*Reversing*: Right mouse click on a function in Quick Access opens the **Context Menu** with the option **Maximize the Ribbon**.

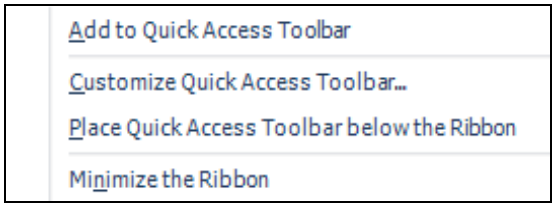

Add to Quick Access Toolbar
Customize Quick Access Toolbar...
Place Quick Access Toolbar below the Ribbon
Minimize the Ribbon

Fig. 2: Context Menu of the functions on the ribbon

***Procedure: Extend Quick Access (Option* Quick Access Toolbar)**

Copied into Quick Access

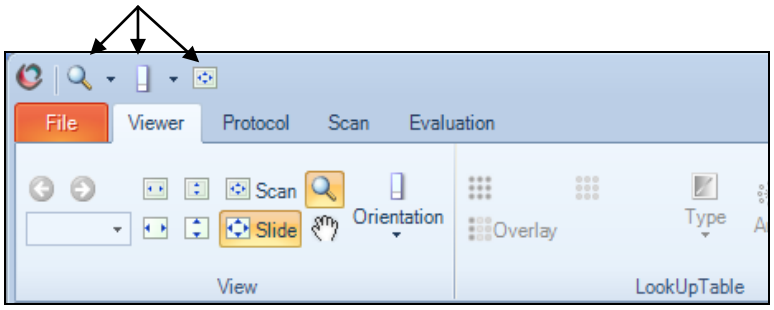


Fig. 3: Quick Access and Ribbon

- ❑ Click with the right mouse button on the desired function on the **Ribbon**. The relating **Context Menu** (Fig. 2) is opened.
- ❑ Click on **Add to Quick Access Toolbar**. The relevant function is copied into the **Quick Access** (Fig. 3).

Menu Bar

Here are the menus of the program. A click on the menu name activates the menu. The tab of the active menu is highlighted brightly. The tab of the menu **File** is always highlighted in orange.

Fig. 4: Menu Bar

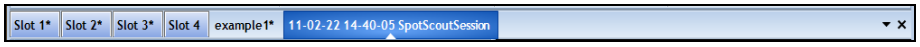
Ribbon

Depending on the menu, the required functions and tools in the form of buttons are arranged and subdivided into functional groups here. You can also move functions which you use frequently to **Quick Access**. See above.

Setting and Evaluation Section

Depending on the menu, the parameters are entered or the displayed image can be analyzed and evaluated here.

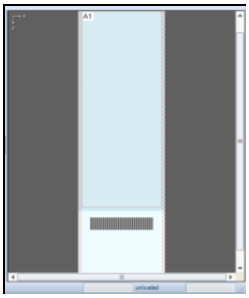
Display Bar for Slots and Loaded Files



Here, the four slot tabs as well as all loaded files are displayed. (A *.ses file always only includes the files of one slot.) The active slot or the active file is highlighted. By clicking on a slot or a file name, the relevant slot or file is activated.
A scan can only be performed in a slot tab. These tabs are always available if a MArS is connected and identified.

The files can also be selected via the drop-down menu of the **Display Bar** <▼>. The active file is closed via <**x**> in the Display Bar.

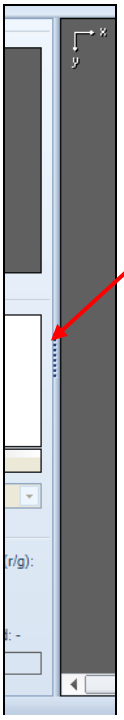
Slide Display



In this section, the scanned or loaded image is displayed. The following scroll and zoom functions are available if the zoom function is active:

Scroll vertically	Moving the mouse wheel
Scroll horizontally	Moving the mouse wheel with pushed <**Shift**> button
Enlarge/ reduce image	Moving the mouse wheel with pushed <**Ctrl**> button
Select and zoom detail	Draw a rectangle with the mouse. The selected detail is zoomed to the size of the slide display.

Splitter Bar



The splitter bar between **Slide Display** and the **Setting and Evaluation Section** can be moved randomly. For that purpose, the splitter bar is provided with a **marking** in the middle to be dragged with the mouse or to be clicked on.

Move	Click on the marking of the splitter bar and move the splitter bar in the desired direction with the mouse button being pushed
Maximize	A click on the marking of the splitter bar enlarges the image display to the total width of the program window
Restore	Double-clicking the marking of the splitter bar restores the original division between the two sections. In the maximized state, the splitter bar is on the far left at the edge of the program window.

On the tabs **Histogram** and **Scatter Plot** in the menu **Evaluation** (only ***SpotScout Pro***), clicking on the **Splitter Bar** has the effect that the image display is faded out in favor of the result display.

Status Display

Status display for the scanner as well as for the analyzing processes like <**Find Grid**> and <**Analyze**>.

Progress Bar of the Selected Slot

It displays the progress of the current action (like e.g. scanning) in the active slot in %.

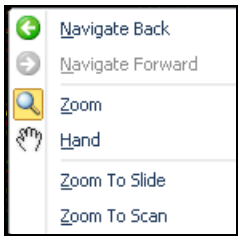
Loading Status

Unloaded	The **MArS** loading table is moved out.
Empty	The **MArS** loading table is in scan position and contains no slides (sample carriers).
Loaded	The **MArS** loading table is in scan position and includes one slide in the displayed slot (sample carrier).
Barcode	If the barcode is read it replaces the loading status.

Global Progress Bar

Displays the progress of the processing of a protocol in % of the total time (see menu **Protocol**).

Context Menu of the Slide View Main Window:



A click with the right mouse button on the **Slide View** opens the **Context Menu** with the following functions:

Navigate Back	The program returns to the last navigation setting (e.g. zoom setting)
Navigate Forward	Proceeds by one navigation setting
Zoom	Activates the zoom function
Hand	Activates the hand function
Zoom to Slide	Zooms to the complete slide
Zoom to Scan	Zooms to the complete scanned image

Keyboard Control

The program can also be controlled via the keyboard:
Pushing the <**Alt**> button indicates the letter to perform a function.

For the options of the drop-down menu, *the* letter which performs the option is underlined.

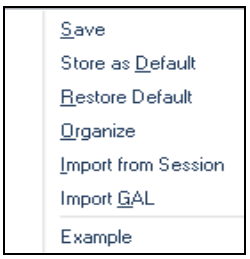
Default Protocol

A default protocol for all parameter settings is implemented in the program which the user can define according to his needs. It must only be observed that there is only one single default file.

There are two basic possibilities for a modification of the default protocol:

- Modification of individual parameter groups: Every parameter group in the menus Scan and Evaluation has a drop-down menu **Presets**. Here, the relevant parameter group can be saved in the default file. Thus, only the relevant parameters are overwritten, the remaining data remain unaffected.
- Modification of the complete default protocol is performed if you save a protocol in the menu **Protocol** as default file, since then the associated settings are saved together with the protocol steps.

As standard, the default file is loaded if a new session is opened (<**New Scan Sessions**>).



Drop-Down Menus Presets

In the drop-down menus **Presets** there is the possibility to save the current settings of the associated parameter group and to load or to delete settings already saved.

Save	Saves the parameter group under a user-defined name.
Save as Default	Saves the parameter group in the available default file (see above).
Load Default	If you have overwritten a default file, you can restore the previous state of the default file with this command.
Organize	Lists the available parameter files in this parameter group. Here, you can delete files.
Import from Session	Here, you can read in parameters of a session file. For that, the available *.ses files are displayed.
Import GAL	This option is only available for grid settings. Here, a GAL file with a grid structure can be loaded.
xxx	In the lower part of the window, the available presets of the relevant parameter group are displayed.

3.1.3. Program Structure

The program is subdivided into several menus which refer to special program functions:

Menu File

It includes

- Functions for loading and saving of files,
- Function for modifying program settings as well as
- Information and documentation functions.

Menu Viewer

In the menu **Viewer**, the loaded or scanned images are displayed, in which you can navigate, zoom and analyze spots with the different functions. A histogram shows the color value distribution of the complete image.

Menu Protocol

In the menu **Protocol**, the steps for a session (from the parameter input to scanning and evaluation) are defined as a **Protocol Flow** and the complete process is started. The parameter input itself is done in the menus **Scan** and **Evaluation** (only ***SpotScout Pro***) or by loading saved protocols in the menu **protocol**.
For routine analyses, protocols saved by means of **Quick Function** can be loaded with corresponding scan and grid parameters and the scan and evaluation process can be started.

Menu Scan

Here, the **Scan Parameters** as well as the **Grid Structure** for the slide (sample carrier) are defined. A single slot can be **scanned** with the parameters defined here or be tested with a **Preview Function**.

Menu Evaluation (only *SpotScout Pro*)

Before scanning, the **Evaluation Parameters** are defined in the evaluation menu. After scanning, the image is analyzed and evaluated here. The results are available as **Result Table** (GPR format). The values of the result table can be directly assigned to every spot in the slide view. Besides, **Regression Analyses** with random value pairs can be performed and displayed as **Scatter Plot**. Additionally, a report on the quality of the evaluation can be created.

3.1.4. File Formats

SES Files

Files with the ending .ses are created by ***SpotScout***. Here, all information, program and scan settings as well as evaluation settings and results (only for ***SpotScout Pro***) are saved per slot. Additionally, the image files are saved as TIF files – corresponding to the number of images and used filters. They have file names which correspond to the associated *.ses file. When loading a *.ses file, the associated TIF files are also loaded.

To prevent the destruction of the relationship of these files, you should not modify the names of the files and copy all related files when copying.

TIF Files

The scanned images are saved as *.ses files and associated TIF files. It is also possible to load individual TIF files (16 bit grey scale images), even if they originate from other scanners. In this case, ***SpotScout Pro*** only needs the information included in the TIF file.
You can also save a session as TIF file. In this case, only image data are saved, all other data get lost.

GAL Files

GAL files (GenePix® format) include grid definitions for slides. They can be directly loaded in the menu **Scan** and replace a manual creation of the grid structure.

GPR Files

The results are given in GPR file format (GenePix® Format). They include image data and analysis data. In this format, the evaluation results of ***SpotScout Pro*** are given.

SEP and SLT Files

In this format, protocols are saved.

HTML Format

The reports of ***SpotScout Pro*** are saved in HTML format.

BAK Files in case of Computer Crash

In case of a computer/software crash, you are prompted to save the current session. In case of an inconsistency of the new file, the previous session is saved as backup (file extension *.bak).

3.2. Menu File

After the selection of the menu **File**, the menu window with the menu options (on the left) and the option **Recent** (last used files and directories) is displayed (see Fig. 5).

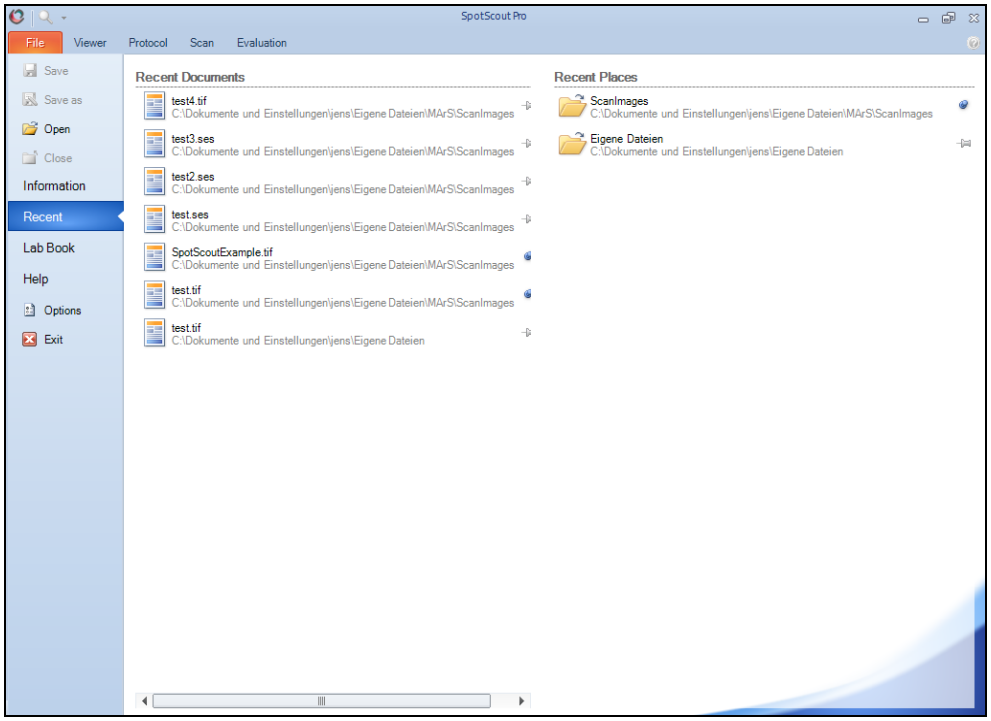
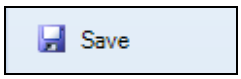


*Fig. 5: Menu **File** with options and default setting **Recent***

3.2.1. Save

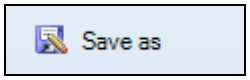


A click on <**Save**> saves the session or image file active in the menu **Viewer**.
If a *.tif file is active, the selection window **Save as** always appears at the function <**Save**> to define the name and to select the file format. Loaded *.tif files can also be saved as *.ses files. In this case, they also include the additional information of the session.

If the opened file is a *.ses file, the available file is overwritten with the changes.

Default setting for the saving of file images is
My Files\MArS\ScanImages.

3.2.2. Save As



This option saves the session or image file active in the menu **Viewer** under a new name to be entered.
The following settings have to be considered and made:

- ❑ Enter the desired **File Name**.
- ❑ Set the desired **File Type** (*.tif or *.ses). *Please observe the explanations on the file types in chapter 3.1.4.*
- ❑ Set the desired memory **mode**:

  Single TIFF: Every scan image (e.g. per **ScanSetting**, per area) is saved as individual *.tif file.

  Multi TIFF: All images are saved in a *.tif file.

  Multi TIFF per Area: All images per area are saved in a *.tif file.
- ❑ Enter the desired **Orientation** of the image. For that, click on the arrow button next to **Orientation**. A selection list opens where the orientation of the sample carrier is pictorially represented.

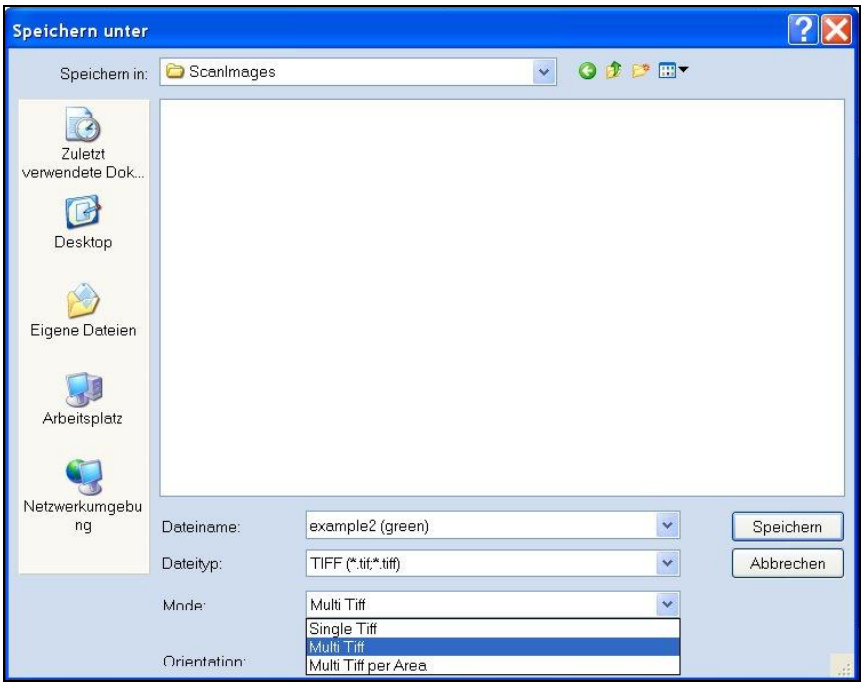


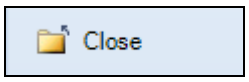
*Fig. 6: Dialogue window **Save as***

3.2.3. Open

The default path for the opening of image files is:
My Documents\MArS\ScanImages.
This path can be modified like in the ***Windows Explorer***.
Open file:

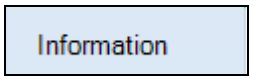
- ❑ Select the desired path.
- ❑ Select the desired file type **(*.ses**; ***.tif** or ***.***).
- ❑ Select the desired file name and click on <**Open**>.

3.2.4. Close



By clicking on <**Close**>, the file currently active in the menu **Viewer** is closed. If the data have not yet been saved or if you have made modifications you are asked whether you want to save the data.

3.2.5. Information



By clicking on <**Information**>, the program version of ***SpotScout*** or ***SpotScout Pro*** is displayed.

3.2.6. Recent

By clicking on <**Recent**>, the documents (column **Recent Documents**) and directories (column **Recent Places**) opened recently are displayed (see Fig. 5).

- ❑ If you click on a document in the column **Recent Document**, the relevant image file is opened and displayed in the menu **Viewer**.

- ❑ If you click on a directory in the column **Recent Places**, here the relevant directory with the files included therein is displayed. Here, you can select and load a file. The file is displayed in the menu **Viewer**.

Permanent Display
The displayed files and directories are provided with one pin each, displayed as loose pin . By clicking on a pin, the file or the folder are displayed permanently even if it has not been opened for a long time. This is marked symbolically by an inserted pin (see Fig. 5).

3.2.7. LabBook

In the **LabBook**, all processes of ***SpotScout*** are protocolled and saved.

Lab Book

After the selection of this option, the protocol is displayed with date, time and process. No process can be deleted or modified.

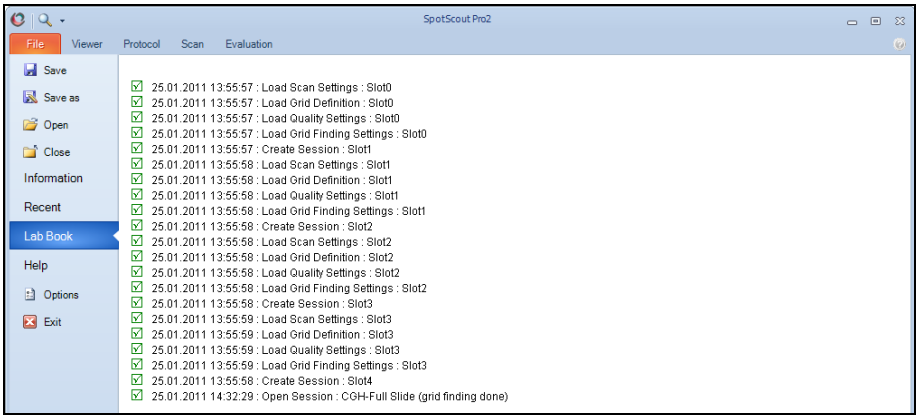

SpotScout Pro2
File
Viewer
Protocol
Scan
Evaluation
Save
Save as
Open
Close
Information
Recent
Lab Book
Help
Options
Exit
25.01.2011 13:55:57 : Load Scan Settings : Slot0
25.01.2011 13:55:57 : Load Grid Definition : Slot0
25.01.2011 13:55:57 : Load Quality Settings : Slot0
25.01.2011 13:55:57 : Load Grid Finding Settings : Slot0
25.01.2011 13:55:57 : Create Session : Slot1
25.01.2011 13:55:58 : Load Scan Settings : Slot1
25.01.2011 13:55:58 : Load Grid Definition : Slot1
25.01.2011 13:55:58 : Load Quality Settings : Slot1
25.01.2011 13:55:58 : Load Grid Finding Settings : Slot1
25.01.2011 13:55:58 : Create Session : Slot2
25.01.2011 13:55:58 : Load Scan Settings : Slot2
25.01.2011 13:55:58 : Load Grid Definition : Slot2
25.01.2011 13:55:58 : Load Quality Settings : Slot2
25.01.2011 13:55:58 : Load Grid Finding Settings : Slot2
25.01.2011 13:55:58 : Create Session : Slot3
25.01.2011 13:55:59 : Load Scan Settings : Slot3
25.01.2011 13:55:59 : Load Grid Definition : Slot3
25.01.2011 13:55:59 : Load Quality Settings : Slot3
25.01.2011 13:55:59 : Load Grid Finding Settings : Slot3
25.01.2011 13:55:58 : Create Session : Slot4
25.01.2011 14:32:29 : Open Session : CGH-Full Slide (grid finding done)

Fig. 7: Display of a laboratory protocol

3.2.8. Help

Help

After the selection of <**Help**>, a window with several options is opened.

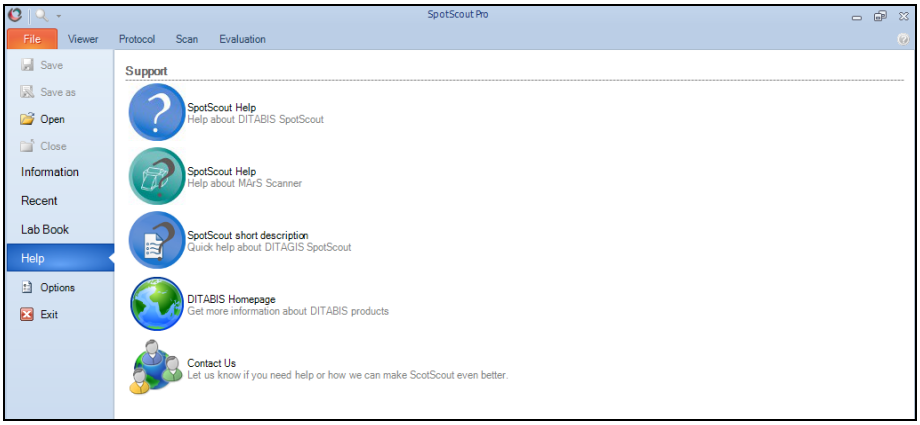


Fig. 8: Menu File

***SpotScout* Help.** Displays the available manual.

***SpotScout* Help MArS.** Displays the **MArS** manual.

SpotScout* short description.** Opens the short description of ***SpotScout.

Ditabis Homepage. The web browser is started and the DITABIS homepage is opened.

The web browser is started and the contact page of the DITABIS homepage is opened.

3.2.9. Options

Options

After the selection of <**Options**>, a dialogue window with two submenus is opened:

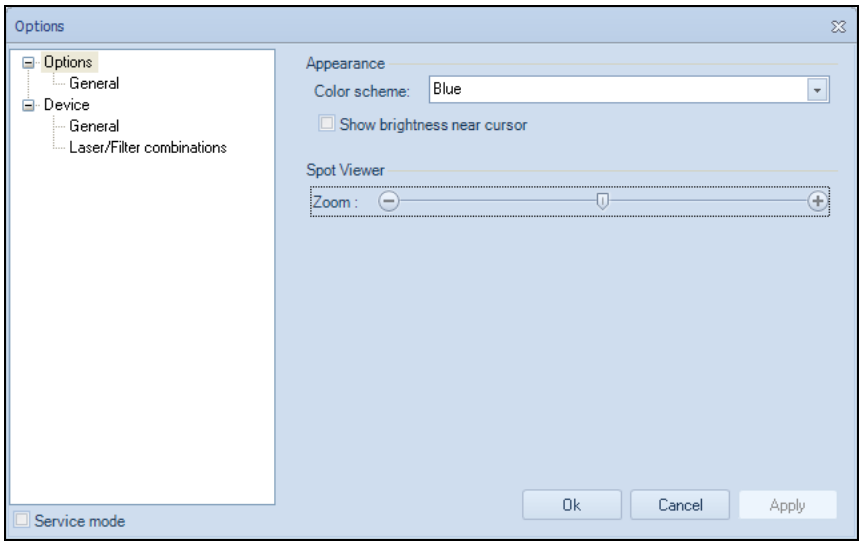


Fig. 9: Menu Options, submenu Options | General

Submenu Options | General

In this submenu, the general appearance (**Appearance**) of ***SpotScout*** and the size of the spots in the **Spot Viewer** (Menu **Viewer a**nd in the menu **Evaluation**, tab **Scatter Plot** and **Evaluation Table**) can be modified:

Appearance

Color scheme	Here, the color scheme can be selected (blue, black, silver)
Show brightness near cursor	If the control box is activated, the brightness values are displayed in the menu **Viewer** next to the cursor.

Spot Viewer

Zoom	With the slider control, the spot size displayed in the **Spot Viewer** can be modified. See chapter 3.3.5

By clicking on <**Ok**>, the settings are saved.

Submenu Device | General

Device Information Here, the **Firmware Version**, the **FPGA Version** and the **Serial Number** of the scanner are displayed if the **MArS** Scanner is connected and switched on.

Preview scan Here, the scanner resolution for the **Preview Scan** can be selected (spot size).

Submenu Device | Laser/Filter Combination

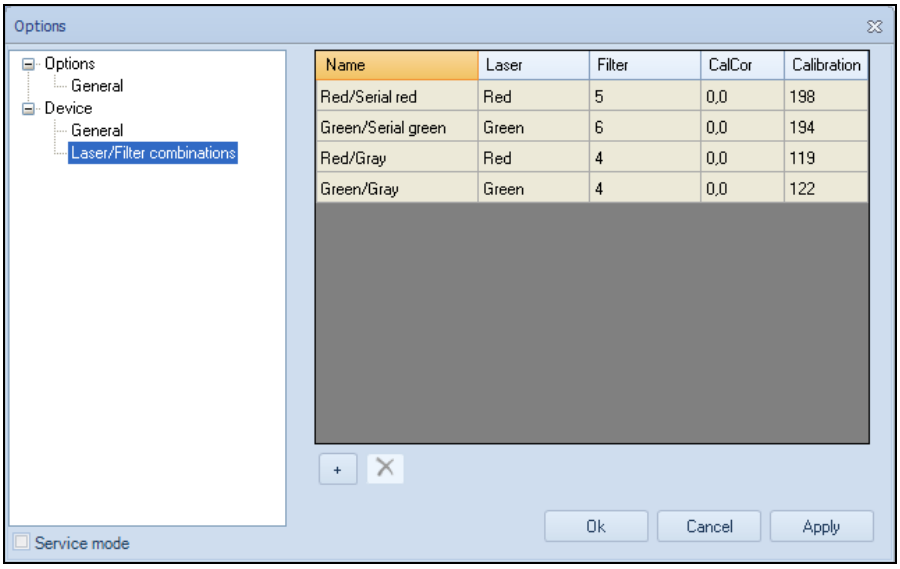


Fig. 10: The four preset laser/filter combinations

The four preset laser and filter combinations (see Fig. 10) are displayed.

Meaning of the individual columns:

Name	Name of the laser/filter combination.
Laser	The possible values (Red/Green) depend on the desired laser [Red (635 nm) or Green (532 nm)].
Filter	Filter position on the filter wheel in **MArS** (see Fig. 11). Possible values are 1...8.
CalCor	Calibration correction value. At the delivery of the device it is "**0**" = no correction. For user-defined filter settings, a deviating value can be entered here, which affects the value in the column **Calibration**. Please note that the top value is 255 counts there.
Calibration	This is the calibration value individually identified for your device. It can be between 0 and 255.

Add Individual Laser/Filter Settings

You can add any individual settings according to the arrangement of the filters in the filter wheel of **MArS**.

Procedure

- ❑ Click on the <**+>** button. A new line is added to the available table.
- ❑ Enter the parameters of the additional filter pursuant to the above description.
- ❑ Confirm the entry by clicking on <**Apply**>.
- ❑ For more filters, repeat this process accordingly.
- ❑ To save the modifications, click on <**Ok**>.

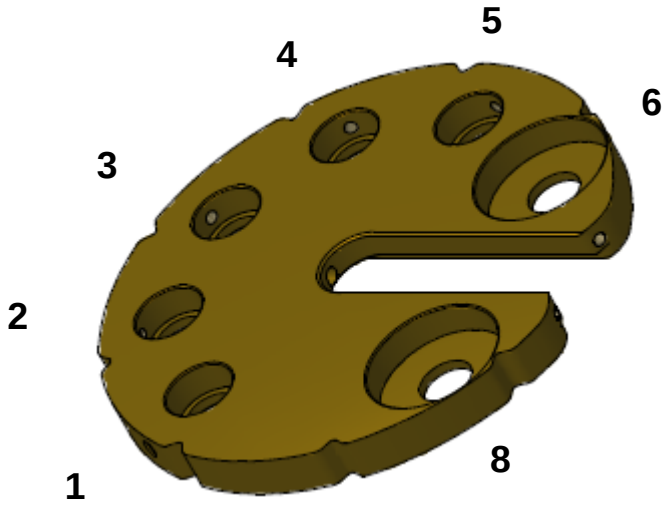


Fig. 11: Filter wheel with the position numbers for the individual filters

Be careful when handling the setting of the calibration correction factors: Even minor modifications of e.g. 1% can increase the measured fluorescence measuring signal by 6 to 10 %.

3.3. Menu Viewer

The menu **Viewer** is the start page of the program as well as the display page for a loaded file or a scanned slide.

The menu window is subdivided into the following sections:

- **Ribbon** with all tools for image setting and navigation
- **Display bar for loaded files and slots**
- **Setting and evaluation section** with **navigators**, **histogram** and **Spot Viewer**
- **Slide View** with display of the scanned or loaded image.

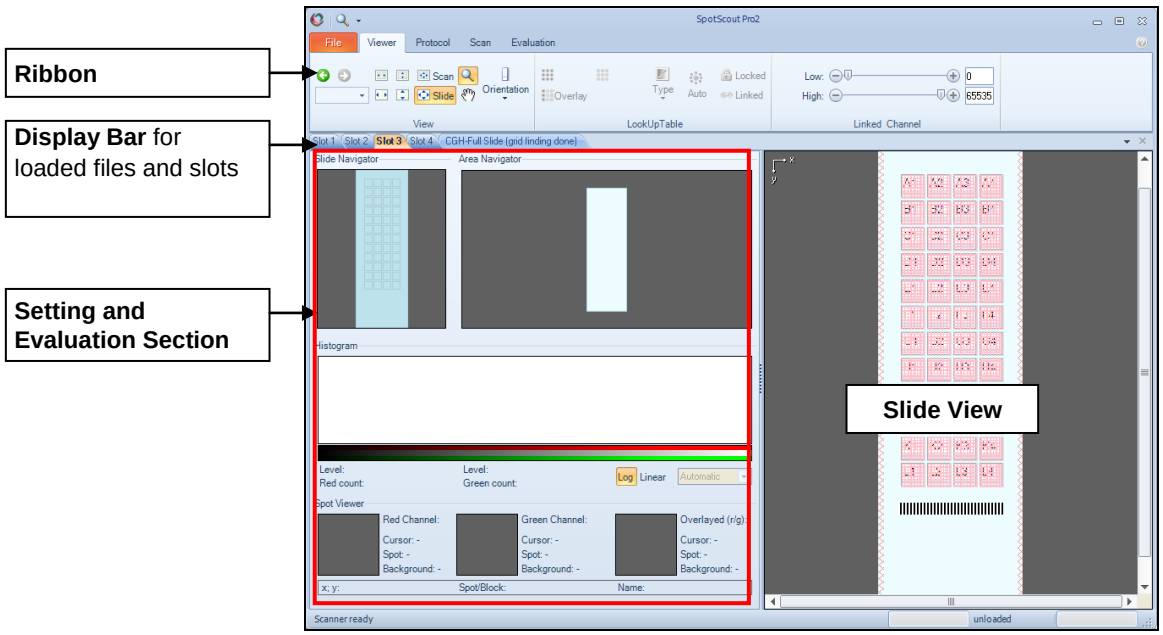


*Fig. 12: Menu **Viewer**, setting: slide - total view*

3.3.1. Overview

Via the **Slide Navigator** and the **Area Navigator**, desired image details for the **Slide View** can be selected and enlarged or reduced. Likewise, the **Slide View** itself can be zoomed (see chapter 3.1.3). These settings mirror themselves in the navigator windows and show you the image detail with surroundings (**Area Navigator**) within the total view of the slide (**Slide Navigator**).

The **Histogram** shows the brightness distribution of the total image as well as the number of counts at the cursor position in the image or in the section you have highlighted in the histogram with the cursor.

The **Spot Viewer** indicates the spot and background values for the spots on which the cursor is placed. A setting of the size of the indicated spots can be made in the menu **File | Options | General** (3.2.9).

3.3.2. Slide Navigator

The **Slide Navigator** shows the total view of the slide, the grey frame shows a block on the slide.

- ❑ Clicking on the block sets the zoom on the block in such a way that only the block is indicated in the **Slide View**.

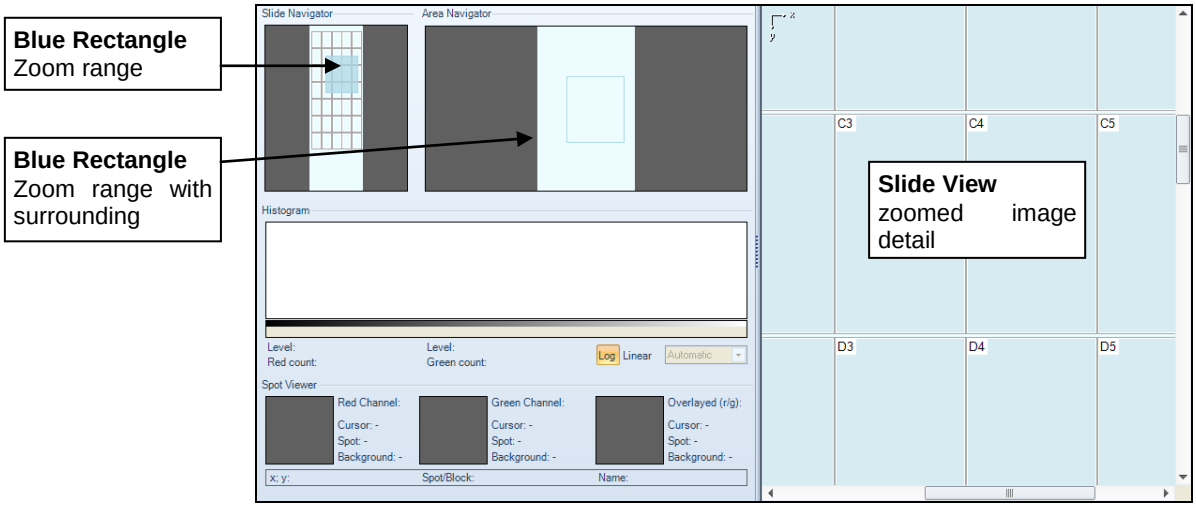


*Fig. 13: Selection in **Slide Navigator** and display in **Slide View***

Set and Move Zoom Range

The blue rectangle is the range which is currently displayed in the main window.
You can define a random blue rectangle in the **Slide Navigator** and with that define the zoom range for the main window and move it afterwards. At the same time, the selected range is displayed in the **Area Navigator** together with the surrounding.

- ❑ Drag a rectangle in **Slide Navigator** with the mouse with the button <**Strg**> being pushed. According to the drawn rectangle, the corresponding range is zoomed in **Slide View**.
- ❑ To move the zoom range, click on the blue rectangle and drag the rectangle in the desired direction with the mouse button being pushed.

3.3.3. Area Navigator

In addition to the selected image detail, the **Area Navigator** shows a range around the displayed image detail (see Fig. 13).

Set and Move Zoom Range

- A random rectangle can be drawn with the mouse in the **Area Navigator**. Accordingly, the display in **Slide View** changes.
- To draw a range within the blue frame, push the button <**Strg**> and draw the desired rectangle with the button <**Strg**> being pushed.
- The “dragging” on one side of the frame enlarges or reduces the detail in the main window.
- To move the zoom range, click on the blue rectangle and drag the rectangle in the desired direction with the mouse button being pushed.

3.3.4. Histogram

The histogram illustrates the distribution of the color values of the complete image. In order to visualize the distribution, the Y-axis is displayed logarithmically (default). You can also change to linear illustration. Depending on the setting either the red or the green or both channels (see chapter 3.3.7) are displayed at the same time (see Fig. 14). For the relevant cursor position, the corresponding values (**Level**, **Red count**, **Green count**) are displayed numerically. A random range can be dragged with the mouse. Thereby, the values for this range (**Level from ... to**; **Red count**; **Green count**) are shown. The selected range is displayed in orange. If you push and hold the button <**Strg**>, the percent values instead of the absolute values for the red and/or green channel are displayed (see Fig. 14).

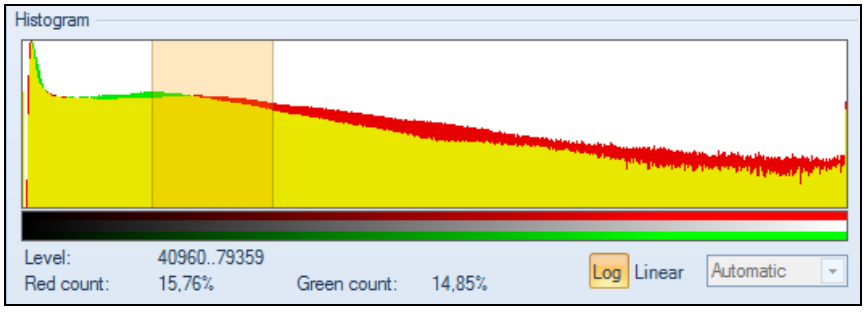


Fig. 14: Histogram illustration with selected range

The histogram also shows whether the scanned image is overexposed or underexposed. If the values on the left side of the histogram are too high, the image is underexposed, if peaks appear on the right side of the histogram, the image is overexposed.

3.3.5. Spot Viewer

The **Spot Viewer** shows the extreme enlargement of the spot which is in **Slide View** below the cursor. At the same time, the intensity value is displayed at the cursor position. After evaluation, the brightness of the spot and the background for every channel (**Red Channel**, **Green Channel**, **Overlayed**) is displayed. Below there are the values for the X-Y-position of the cursor on the slide.

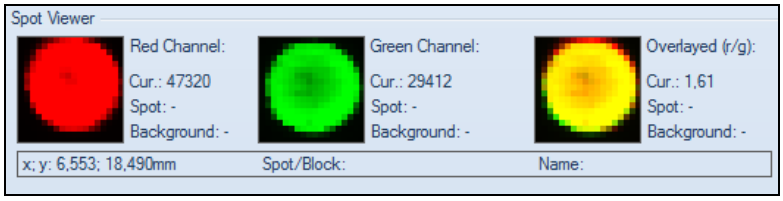


Abb. 15: Spot Viewer

3.3.6. Ribbon – View Functions

Goes to the previous or next view.

In this drop-down menu there are preset zoom factors. Next to these values, a random zoom factor up to 10000% can be entered. The input must be confirmed with <**Enter**>.

Those three functions are related to the scanned image:

The loaded image is enlarged until the width or the height of the scan exactly fills in the main window.

The loaded image is enlarged until the scanned part is completely visible in the main window.

Those three functions are related to the slide

The slide is displayed in full width or in full height.

In the main window, the zoom factor is set so that the slide is entirely visible.

Zoom tool. If this tool is activated, a section can be selected in the Slide View with the mouse which then is enlarged accordingly (see chapter 3.1.2).

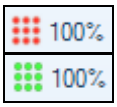
Hand tool. If this tool is active, the image can be moved in the **Slide View** so that another image detail is displayed. For that, you have to click on the image and drag it.

In the drop-down menu **Orientation**, the orientation of the slide can be changed in steps of 90°.

As saved

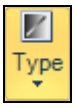
With the option **As saved**, the image is displayed in the saved original position.

3.3.7. Ribbon – Lookup Table Functions



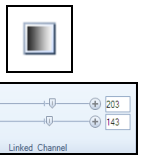
Selection of the displayed channel (red or green). In case of more than one scan image, the desired image can be selected via a drop-down menu <▼>.

The selected images are overlaid (red and green channel).

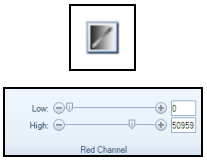


In this drop-down menu, the type of the lookup table can be selected: **Brightness/Contrast**, **Ramp** and **False color.**

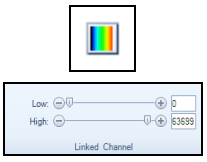
Via the slider control (on the right), the parameters can be set. The relevant setting is displayed on the screen. (As long as the slider control is hold with the mouse, the image is displayed in the preview mode.) If the function **Linked** is active, red and green are set via one slider control, otherwise via separate slider controls.



Option **Brightness/Contrast**: Via the slider control, **Brightness** and **Contrast** of the image can be set.



The option **Ramp** is the default setting. At **Low**, the lowest and at **High**, the highest intensity value for the desired illustration is entered. Between those two values, the illustration from black to bright (color) takes place. Values below the low value are illustrated black, values above the high value are illustrated bright.



Option **False color**: The intensity values are shown on the color scale. See explanations for the option **Ramp**.

Automatic setting of the **LookUp Table**.

If this function is active, the **Lookup Table** settings for all sessions are taken over.

If this option is active, the red and the green channel are set via joint slider controls. Otherwise, different slider controls are displayed for the two channels.

3.4. Menu Protocol

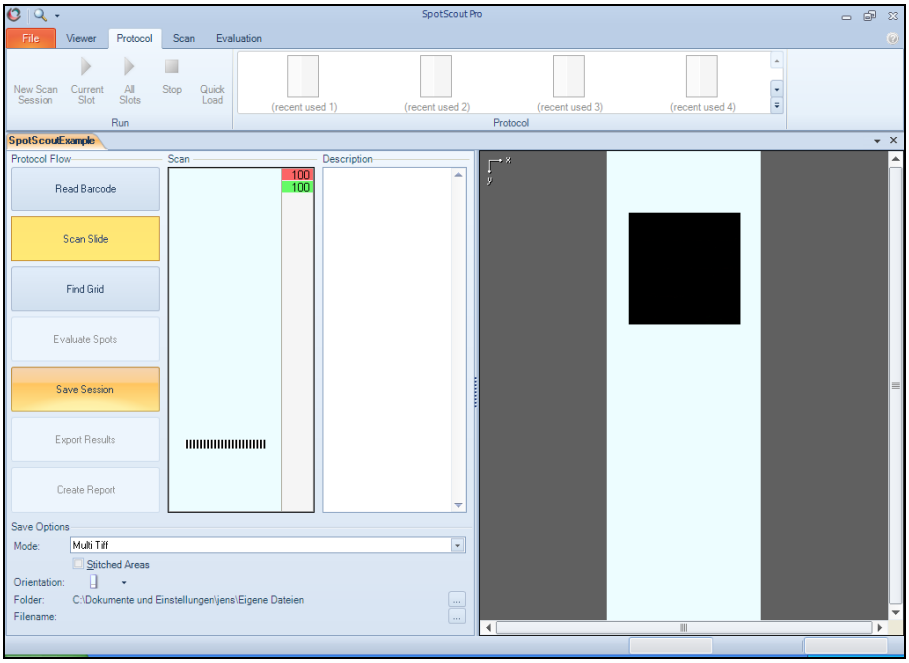


*Fig. 16: Menu **Protocol: Protocol Flow** in the left section, above: file name*

3.4.1. Overview

Display of the defined steps in a Protocol Flow and Start

In the Protocol Flow, the steps to be processed are defined. The steps include the settings which are made in the menus **Scan** and **Evaluation** (only ***SpotScout Pro***) for the active slot.
The steps highlighted in yellow are active and are performed. The individual steps of the **Protocol Flow** are displayed as buttons which can be activated or deactivated by clicking.
Next to the step switches, the scan surface and the settings of the active slot are displayed like they are defined by the selected protocol.

Define New Session

Via the button <**New Sessions**>, new sessions in the four slots can be defined.
Then, the parameters for the new session can be entered by loading available protocols or by entering the parameters for the currently active slot in the menus **Scan** and **Evaluation** (only ***SpotScout Pro***).

Saving the Session

In the section **Save Options**, the saving parameters for the session can be defined.

Starting the Scan Process

With the defined settings, the scan process either for the active or for all occupied and defined slots can be started via the button <**Start**>.

3.4.2. Protocol Flow

The meaning of the individual steps:

Read Barcode	Scans the barcode.
Scan Slide	Scans the loaded slots.
Find Grid	Searches the grid structure pursuant to the preset parameters (only for ***SpotScout Pro***).
Evaluate Spots	Performs the evaluation pursuant to the preset evaluation parameters (only for ***SpotScout Pro***).
Save Session	Saves the session under the file name specified in **Save Options**.
Export Results	Exports the evaluation results as GPR file (GenePix® Results). It is filed together with the session file under the same name. The files only differ from each other in the ending.
Create Report	Creates a HTML report about the quality of the evaluated grid structure. The report is filed together with the session file under the same name. The files only differ from each other in the ending.

3.4.3. Section Save Options

*Fig. 17: Section **Save Options***

Here, it is specified how the scanned image is to be saved.
*Please note that a *.ses file only includes the data of one slot each!*

Mode	Selection of the file format in which the image (not the session) is to be saved. Multi TIFF, Single TIFF and Multi TIFF per area can be selected. See chapter 3.1.4.
Stitched Areas	If the checkbox is activated, the section between the areas is filled black (intensity 0) and one single image is created per **Scan Setting**.
Orientation	By clicking on the arrow button, the drop-down menu is opened in which it is defined with which orientation the image is to be saved.
Folder	Specification of the folder in which the file is to be filed. Default setting is **My Files\MArS\ScanImages**.
File Name	Specification of the file name. By clicking on the arrow button, the dialogue window **Auto Filename** for the input of the parameters for the file name opens (Fig. 18). After the saving of the entries, the file name is displayed.

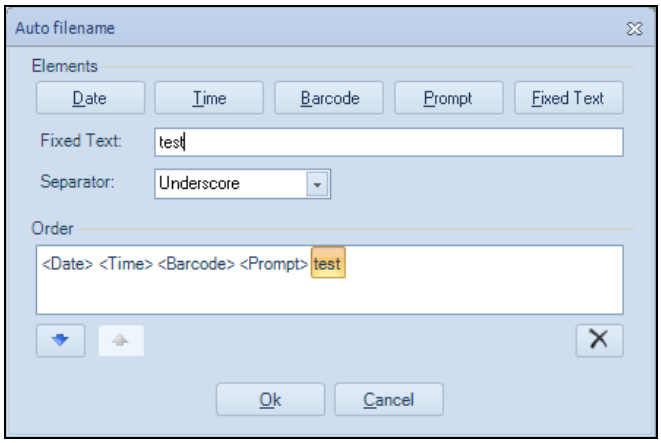


*Fig. 18: Dialogue window **Auto Filename***

For the automatic scan process with the **Protocol Flow**, please note that the file names are generated automatically. For the automatic naming, a selection of the following **elements** in random order can be used:
<Date>, <**Time**>, <**Barcode**>, <**Prompt**> and <**Fixed text**>.
As separator, you can use either an underline or a blank.

Procedure

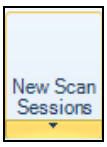
- ❑ Define the **Separator**.
- ❑ If you want to use a specified text in the file name, enter it in the box **Fixed Text**.
- ❑ Then, click on the element buttons in the desired order. The selected elements are then displayed in the box **Order**.
- ❑ To change the order, you can use the arrow buttons below the box **Order** (explanation see below).
- ❑ To delete an element, click on < >.
- ❑ By clicking on <**OK**> you save the parameters for the file name.

The *Elements* of the automatic file naming in detail:

Date	Uses the current date for the file name
Time	Uses the current time for the file name
Barcode	Uses the barcode for the file name. The barcode is read before the Slide Scan
Prompt	Uses a **Prompt** for the file name. The **Prompt** can then be specified before the processing of the protocol in a pop-up window.
Fixed Text	You can add a fixed text which e.g. characterizes the sample.

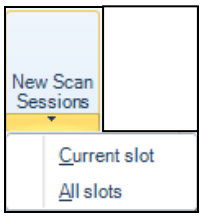
	Moves the element highlighted in the box **Order** forward by one position.
	Moves the element highlighted in the box **Order** backward by one position.
	Deletes the highlighted element.

3.4.4. Define New Session



A new session *for all slots* is defined via the button <**New Scan Sessions**>.

- ❑ Click on <**New Scan Sessions**>. A possibly opened session is closed and a new protocol for all slots is opened.
- ❑ If information of an old session which is not yet saved is available, a save prompt is performed. If applicable, save the data of the last session. Only then, a new protocol for all slots is opened.



A new session *for the active slot* is defined via the drop-down menu of the button <**New Scan Sessions**>.

- ❑ Click on the drop-down menu of <**New Scan Sessions**> and then the option **Current Slot**. A possibly opened session for the active slot is closed and a new protocol for this slot is opened.

3.4.5. Load Protocols

In the ribbon, the protocol files already saved are displayed.
At the first program start, only placeholders for the protocols are displayed (Fig. 19). After the protocol has been saved, the placeholder is replaced as well as the name of the saved protocol is displayed.

Fig. 19: Display of the placeholders for protocols in the ribbon

Fig. 20: Saved protocol

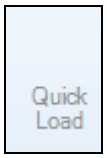
Save/Delete Protocol - Procedure

- Click on the arrow button in the protocol display (see red arrow in Fig. 19). The drop-down menu with the available protocols as well as the options is displayed.
  - For saving, select the option **Save** and then enter the desired file name in the dialogue window.
  - You can also save a protocol as default protocol (Option **Save as Default**). Since there is only one default file (**Default**), you are asked whether you want to overwrite the previous settings in the default file. If you confirm this, the old default file is overwritten with the new files (see also the details on default files, page 15).
  - To delete a protocol file, select the option **Organize**. The window **Organize Protocols** opens in which all protocols are listed. Highlight the protocols to be deleted and click on the button <**Delete**>.

Load Protocol – Procedure

- To load a protocol, click on the desired protocol in the ribbon. The highlighted protocol is loaded for the active slot.

Quick Load Function



Via the button <**Quick Load**> a quick load function for protocols is available to provide several slots with protocols subsequently.

Procedure

- Click on <**Quick Load**>. The quick load function is activated.
- Click on the desired protocol in the ribbon. Then, the selected protocol for the active slot is loaded. At the same time, the program jumps to the next slot and activates it.
- Click on the protocol that you want to assign to this slot. It is loaded and the program jumps to the next slot.
- In this way, you can provide all slots one after the other with different (or identical) protocols.

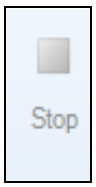
3.4.6. Start/Stop the Processing of the Protocol

The protocol can be started if the protocol steps and the saving parameters are defined.

Start the Processing of the Protocol - Procedure

- ❑ To scan all slots, click on <**Start**>.
- ❑ To scan only the displayed slot, click on the drop-down menu of <**Start**> and select the option **Current Slot**.
- ❑ Then, all steps of the Protocol Flow are processed automatically and – if defined in the **Protocol Flow** - saved. The data are saved per slot in a *.ses file.

Stop the Processing of the Protocol - Procedure



- ❑ Click on the button <**Stop**>. The processing of the protocol is stopped.
- ❑ To proceed with the processing, click on <**Start**> again or select the option **Start | Current Slot** again in the drop-down menu of the button <**Start**>.

3.5. Menu Scan

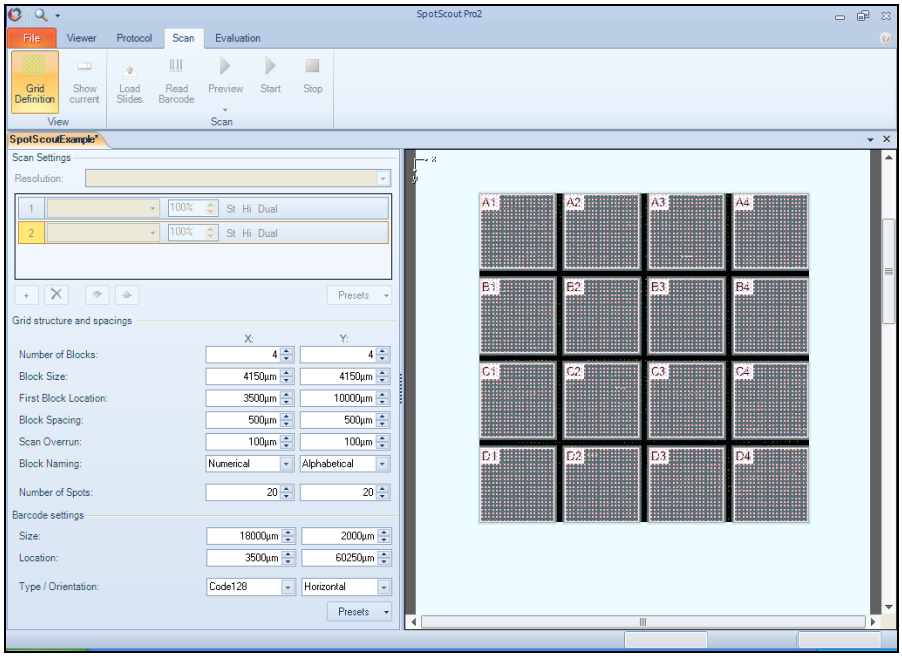


*Fig. 21: Menu **Scan***

3.5.1. Overview

Parameter Input and Grid Definition

In the left section, the parameters for the scanning and the grid structure of the active slot are entered. The parameters can be saved via the corresponding drop-down menus **Presets** and saved parameters can be loaded.

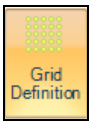
In the slide view, the settings are displayed.

Scanning

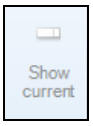
The active slot can be scanned according to the entered settings: As preview (with lower resolution) or as optimal scan. The barcode can also be scanned.

Please note that these scanning functions only refer to the active slot. In the menu **Protocol**, all slots can be scanned one after the other automatically.

3.5.2. View Functions

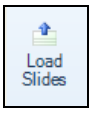


If this button is active, the defined block structure is faded in as half-transparent image in the **Slide View**.



The range which is scanned at the moment is displayed.

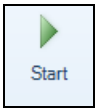
3.5.3. Scan Functions



If **MArS** is closed, the loading table is retracted.

The barcode is scanned in the active slot.

The preview image of the current slide is generated in one or all wavelengths for the active slot with low resolution. The selection can be selected via the related drop-down menu.



Scans the active slot with current settings.

Stops the current scan process.

3.5.4. Section Scan Settings

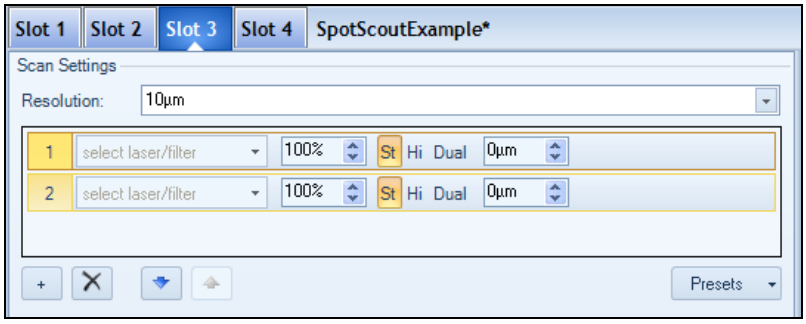


Fig. 22: Scan Settings

The scan parameters for the active slot are displayed. In our example, it is **Slot 3**. These parameters can be saved via the button <**Presets**> or available parameters can be loaded.

The Parameters in Detail:

Displays the resolution (spot size) in µm with which the slide is scanned.

Selection of the laser and filter for the scan process.

Intensity of the laser with which the slide is scanned.

Data transfer via the standard channel

Data transfer via the high channel

Data transfer in *Dual Mode*, *St* and *Hi*

Allows adding more wavelengths.

Removes the currently selected wavelength.

Moves the selected wavelength in the scan sequence down by one position.

Moves the selected wavelength in the scan sequence up by one position.

Setting possibility of the *Defocus* in µm.

Loading / saving of the settings for the scan process. See page 16.

3.5.5. Section Grid Structure

Here, the grid structure and the distances between the blocks are defined.

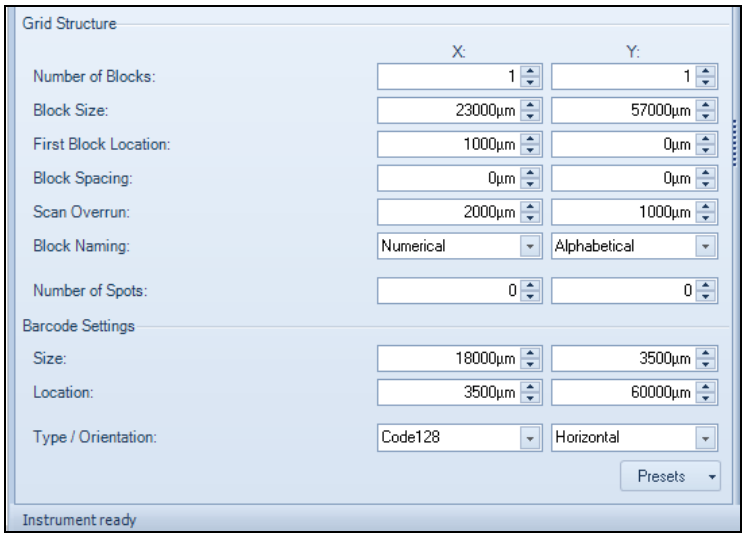


Fig. 23: Grid Structure and Barcode Settings

The Parameters in Detail

Number of Blocks	Number of blocks in *X* – or *Y* – direction.
Block Size	Size of blocks
First Block Location	*X* and *Y* position of the first block
Block Spacing	Distance between the individual blocks.
Scan Overrun	Indicates the scan excess, i.e. how far does the scanner scan beyond the block borders. Tolerances of spotter and insertion tolerances are evened out.
Block Naming	Naming of the blocks. You can select numerical or alphabetical for each the *X* – or *Y* – coordinate.
Number of Spots	Number of spots within a blocks in *X* – and *Y* – direction.
Barcode Settings	
Size	Indication of the size of the barcode
Location	Indication of the position of the barcode
Type / Orientation	Selection of barcode type and orientation
Presets	Loading / saving of the grid structure settings (see details on **Presets** on page 16).

Hints for the Setting of the Grid Structure

- First define the top left block.
- For that, measure the size of this block and enter the values in the line **Block Size**.
- Then measure the position of this block on the slide and enter the values in the line **First Block Location**.
- Measure the distance between the blocks and enter the value in the line **Block Spacing**.
- Enter the number of blocks in X- and Y-direction in the line **Number of Blocks**.

3.6. Menu Evaluation (only *SpotScout Pro*)

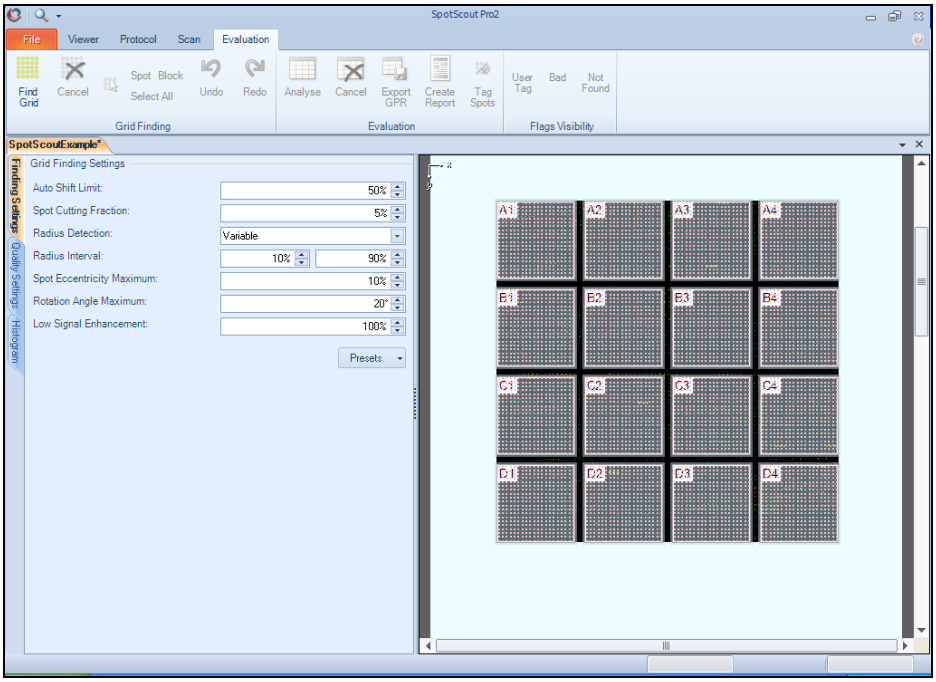


Fig. 24: Evaluation Ribbon: Finding Settings

The menu **Evaluation** is provided with two different functionalities:

- **Parameter input** for evaluation. For that, 2 tabs in the left section are available (**Finding Settings**, **Quality Settings**).
- **Evaluation** after the scan process: Display of the used parameters and display of the evaluation results. For that, more tabs are available in the left section (**Histogram**, **Scatter Plot** and **Evaluation Table**). After evaluation, the parameters for the **Grid Finding** can be changed as well as specific evaluation and analyzing functions can be performed which are available in the form of buttons in the ribbon.

3.6.1. Tab Finding Settings

Parameters for the Finding of the Grid Structure (see Fig. 24).

Auto Shift Limit	Here, the maximum distance of the ideal grid structure is defined within which spots are still searched. 100% corresponds to half the spot distance.
Spot Cutting Fraction	This parameter defines the lower intensity limit. It defines how much background is to be deducted of each spot in percent.
Radius Detection	This parameter defines whether the grid finding is to be performed with a fix or variable radius.
Radius Interval	This parameter defines the range for the spots radii, which is acceptable. 100% corresponds to the radius of the spot.
Spot Eccentricity Maximum	Here, the possible eccentricity of the expected spots, i.e. the deviation from an exact circle is set. A value of 0% means that only perfect circles are accepted as spots. A value of 10% means that also slightly oval spots are accepted. At the maximum input of 100%, even a linear spot is accepted.
Rotation Angle Maximum	Here, the angle is entered by which the grid can be maximally rotated. The smaller the angle entered here, the smaller the searching range of the grid finding function.
Low Signal Enhancement	This parameter influences the depth of focus for lower image signals. High image signals lead to an exact analysis while lower values consume storage space and evaluation time in favor of a high-grade detection of lower spot signals.
Presets	Via the drop-down menu, **Grid Finding** parameters can be saved or saved parameters can be loaded. See details on **Presets** on page 16.

3.6.2. Tab Quality Settings

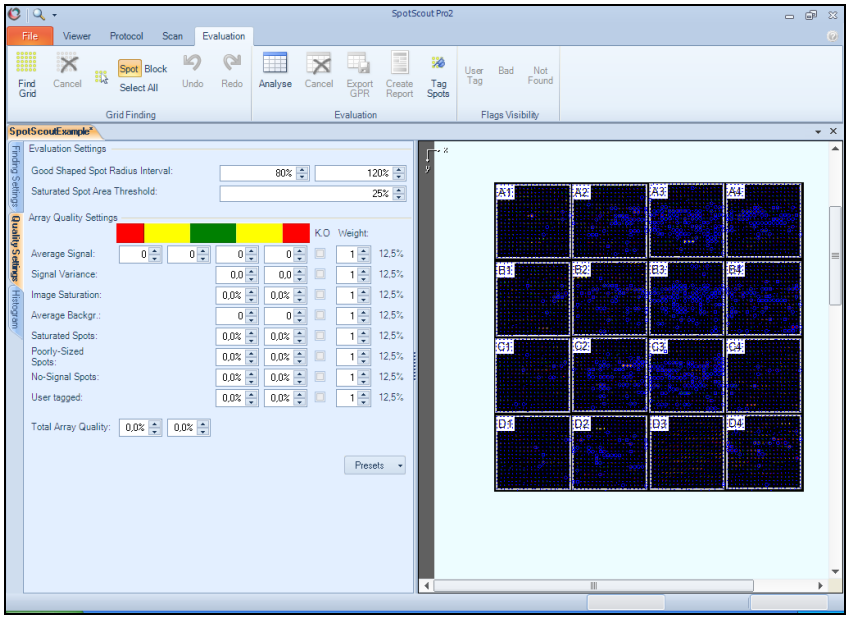


*Fig. 25: Tab **Quality Settings***

Section Evaluation Settings

Good Shaped Spot Radius Interval	With these parameters, the upper and lower limit for the deviation of a spot position from the defined position is set. The distance is entered as percentage of the spot radius median. Values of more than 100% are possible.
Saturated Spot Area Threshold	This parameter defines the limit from which a spot is considered to be overexposed. Here, the percentage of spot pixel is entered which has an intensity value of more than 98% of the maximum intensity.

Section Array Quality Settings

Here, the limits for the evaluation ranges are defined. They are the basis for the **Report**.

In the medium range, values are accepted as OK (“Green”).
This range is followed at both ends by ranges with the evaluation “yellow”. Outside those ranges, the evaluation is „red“.

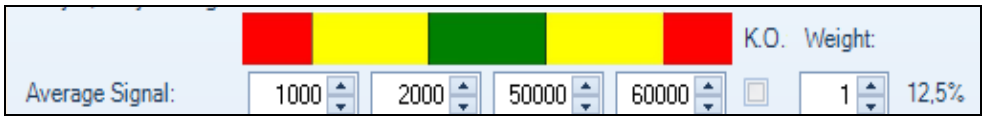


Fig. 26: Scheme Quality Parameter

For the levels as well as for the following parameters, a weight for flowing into the total evaluation and the definition as exclusion criteria can be made. The exclusion criteria say: if the parameter concerned delivers a red evaluation, the chip must be evaluated as being red.

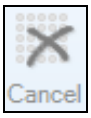
Average Signal	Limits for the average intensity of all spots.
Signal Variance	Limits for the variance of the intensity of all spots.
Image Saturation	Limits for the percentage of the pixels above the overexposure limit.
Average Background	Limits for the average background intensity of all spots.
Saturated Spots	Limits for the percentage of overexposed spots within the relevant spot range.
Poorly-Sized Spots	Limits for the percentage of badly positioned spots within the spot range.
No-Signal Spots	Limits for the percentage of no-signal spots within the spot range.
User tagged	Limits for the percentage of user-defined spots within the spot range
Presets	Loading or saving of parameters. See details on **Presets** on page 16.

3.6.3. Grid Finding Options

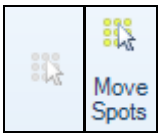
In this section of the ribbon, the options for **Grid Finding** are summarized.

Starts the **Grid Finding**. For that, at least 5 spots must be defined.

By another click on this button, the function is reset. By yet another click, the grid finding is started again.



Cancels the **Grid Finding**.

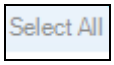


Activates the tool **Move Spot**. Then, click on the button <**Spot**> or <**Block**>. By that, you can move a spot or a spot block or all spots (with the mouse button being pushed).

If you select a range with the button <**Strg**> being pushed, the spots in this range are highlighted and the spots in this range which have already been highlighted remain highlighted.

Tool to move a spot. The tool **Move Spots** must be activated.

Tool to move a spot block. The tool **Move Spots** must be activated.



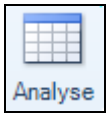
Tool to move all spots together. The tool **Move Spots** must be activated.

Resets the last spot movement.

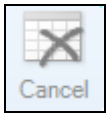
Repeats the last spot movement.

3.6.4. Evaluation Options

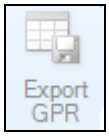
In this section of the ribbon, the evaluation options are summarized.



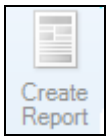
Analyzes the spots within a grid structure.



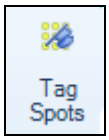
Cancels the grid analysis.



To be able to evaluate the data with other programs, the analyzing result can be exported as *.gpr file (gene pix results).



Creates a HTML Report which describes the quality of the grid analysis. The report is filed together with the session file of the same name.



Tool to highlight spots. Individual spots are highlighted by clicking. A spot group is highlighted by selecting the relevant range with the mouse. If you push the button <**Strg**> during that, spots already highlighted in this range remain highlighted.

3.6.5. Flags Visibility Options

In this section of the ribbon, the options are summarized with which the flags defined by the evaluation parameters can be displayed.

Display of the highlighting symbols for the spots highlighted by the user. See tab **Quality Settings**, parameter **User tagged**.

Display of the highlighting symbols for the spots classified as “bad”. See tab **Quality Settings**, parameter **Poorly-sized Spots**.

Display of the highlighting symbols for the spots classified as no-signal spots. See tab **Quality Settings**, parameter **No-Signal Spots**.

Display of the highlighting symbols for the spots classified as saturated. See tab **Quality Settings**, parameter **Saturated Spots**.

3.6.6. Tab Histogram

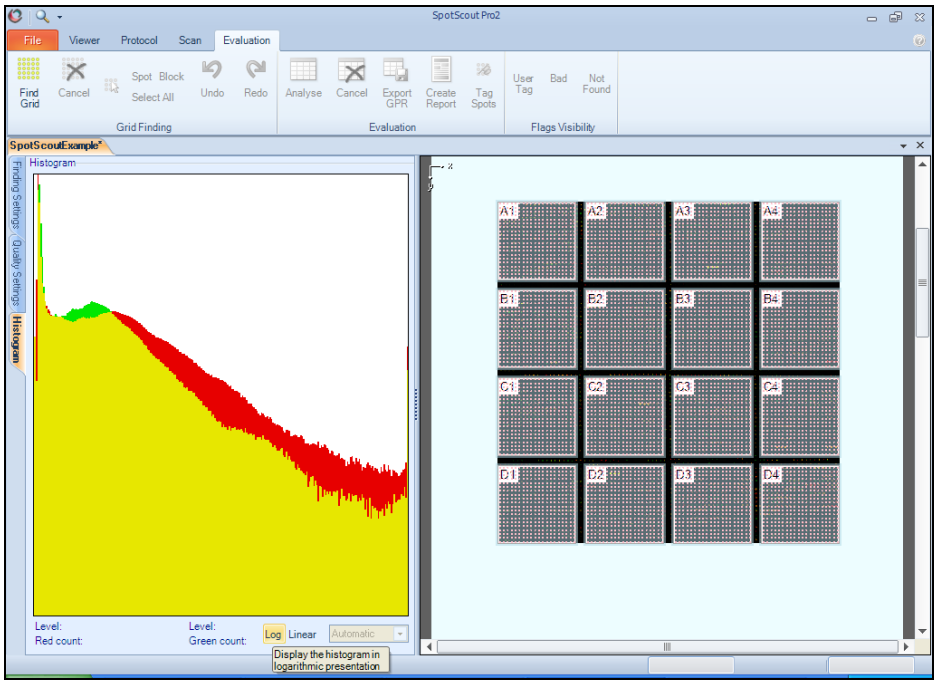


Fig. 27: Evaluation Ribbon: Histogram

Please read description in chapter 3.3.4.

3.6.7. Tab Scatter Plot

After the analysis of the spots, two new tabs are displayed in the left section, **Scatter Plot** and **EvaluationTable**.

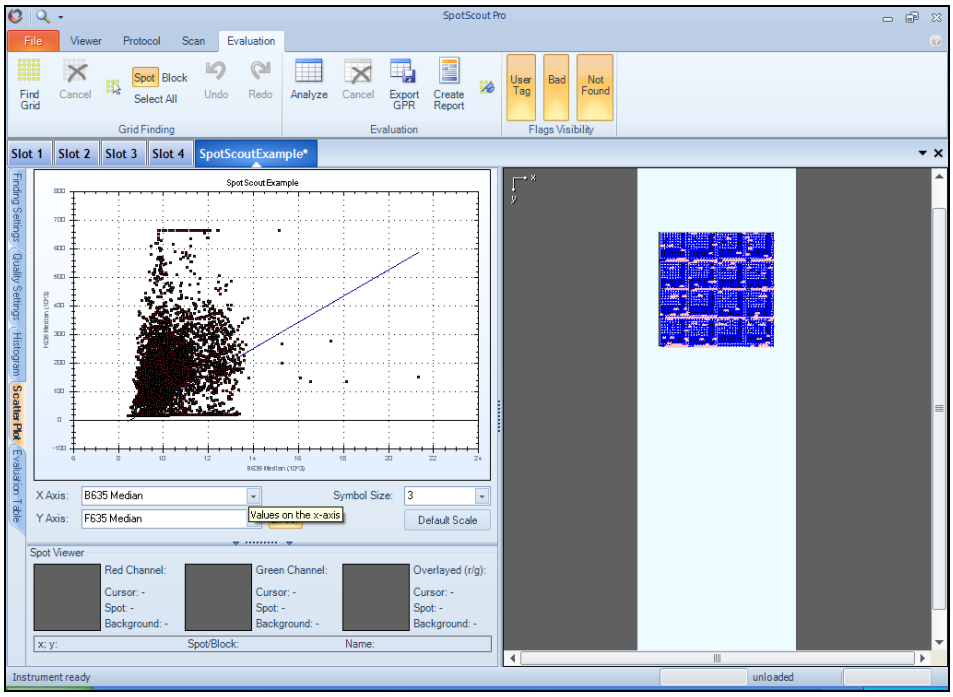


*Fig. 28: Tab **Scatter Plot** with an example evaluation*

In **Scatter Plot**, all features of the spots, like e.g. background intensity of the relevant wavelength can be plotted against each other on the X-axis and on the Y-axis.
The blue line in the plot is a *Regression Line,* which is calculated depending on the selected value pairs.
The symbol size of the displayed spots can be set in the box **Symbol Size** below the Scatter Plot. By clicking on <**Default Scale**>, the original size is restored.
When you move the mouse over a spot, the ID of the spot is faded in via Tooltip and the relevant spot is displayed in the **Spot Viewer**.

3.6.8. Tab Evaluation Table

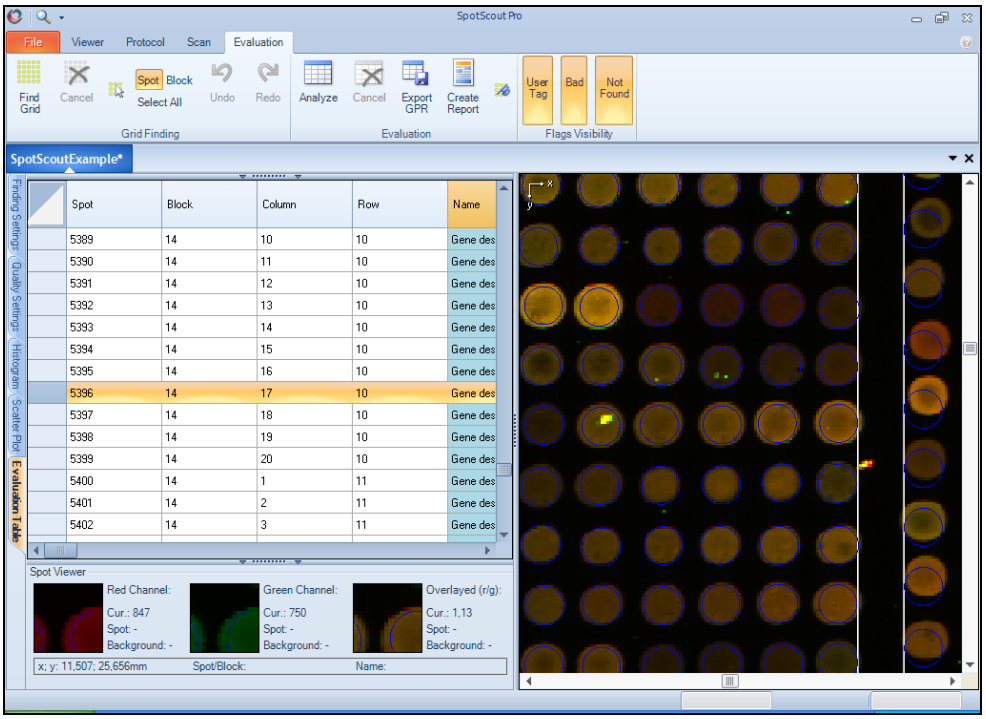


Fig. 29: Evaluation Table

In the **Evaluation Table**, all results of the analysis of the slide are summarized.

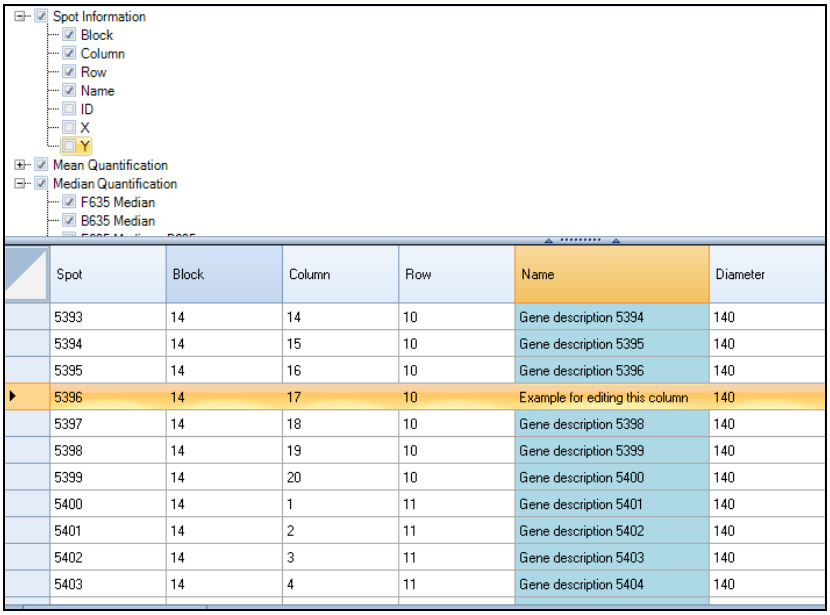
- A double-click on a spot in **Slide View** lets the table jump directly to this spot, e.g. in Fig. 29, spot 5396 has been clicked on.
- A double-click on a spot in the table positions the spot in **Slide View** in the middle of the image.
- A double-click on the splitter bar between **Slide View** and **Table** (see page 14) maximizes the table.

Next to the vertical splitter bar, there are two other splitter bars above and below the table:

- A double-click on the lower splitter bar hides/shows the **Spot Viewer**.
- A double-click on the top splitter bar opens/closes the section where columns displayed in the evaluation table can be selected. Selected columns are highlighted by ticks. Clicking on a checkbox deactivates/activates the relevant column in the table (see Fig. 30).

In Fig. 30, the columns **ID**, **X** and **Y** are faded out. Likewise, the column **Name** of spot 5396 has been changed into an example text. In the **Evaluation Table**, the columns **Name**, **ID** can be individually labeled.

The columns **User Tag**, **No Signal**, **Poor Size** and **Saturated** can also be changed via drop-down menu.



Spot	Block	Column	Row	Name	Diameter
5393	14	14	10	Gene description 5394	140
5394	14	15	10	Gene description 5395	140
5395	14	16	10	Gene description 5396	140
5396	14	17	10	Example for editing this column	140
5397	14	18	10	Gene description 5398	140
5398	14	19	10	Gene description 5399	140
5399	14	20	10	Gene description 5400	140
5400	14	1	11	Gene description 5401	140
5401	14	2	11	Gene description 5402	140
5402	14	3	11	Gene description 5403	140
5403	14	4	11	Gene description 5404	140

Fig. 30: Evaluation Table with edited names

If you move over the lines with the cursor in the table, the relevant spot is displayed in the **Spot Viewer**.

4. Appendix

4.1. Evaluation Table

Columns created by the evaluation algorithms:

Block	Block number of the feature
Column	Column number of the feature (in block)
Row	Row of the feature (in block)
Name	Description field for spot
ID	Description field for spot
X	X-coordinate in µm of the center of the feature-indicator associated with the feature, where (0,0) is the top left of the image
Y	Y-coordinate in µm of the center of the feature-indicator associated with the feature, where (0,0) is the top left of the image
Dia	Diameter in µm of the feature indicator

For each analyzed channel (XXX marks channels wavelength):

FXXX Median	median feature pixel intensity
FXXX Mean	mean feature pixel intensity
FXXX SD	standard deviation of the feature pixel intensity
FXXX CV	Coefficient of variation - is calculated by FXXX SD/ F XXX Median
BXXX	median feature background intensity
BXXX Median	median feature background intensity
BXXX Mean	mean feature background intensity
BXXX SD	standard deviation of the feature background intensity
BXXX CV	Coefficient of variation - is calculated by BXXX SD/ B XXX Median
%>BXXX+1SD	percentage of feature pixels with intensities more than one standard deviation above the background pixel intensity
%>BXXX+2SD	percentage of feature pixels with intensities more than two standard deviations above the background pixel intensity
FXXX%Sat.	Percentage of feature pixels which intensity equals the maximum intensity
FXXX Median – BXXX	the median feature pixel intensity at wavelength with the median background subtracted
FXXX Mean – BXXX	the mean feature pixel intensity at wavelength with the median background subtracted
FXXX Total Intensity	the sum of spot pixel intensities at wavelength Index number of the spot as it occurs on the array
SNR XXX	the signal-to-noise ratio at wavelength, defined by (Mean Foreground 1 – Mean Background 1)/(Standard deviation of Background 1)

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